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OBSERVATIONS ON PHAGOCYTOSIS AND LEUKOCYTIC ACTIVITY IN DIPHTHERIA-CARRIERS *

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The following experiments on phagocytosis in diphtheria-carriers were made in the hope that some light might be thrown on the mechanism whereby the bacilli are removed.

Opsonic preparations were made in the usual way. Suspensions of the leukocytes of normal persons and of diphtheria patients and carriers were made so that all should contain approximately the same number of polymorphonuclears. Various strains of diphtheria bacilli, including the strain isolated from the patient whose serum was being tested, were used; all gave similar results. The opsonic index, the cytophagic index, or the activity of the patient's leukocytes as compared with a normal person's, and the opsonic-cytophagic index, or the cytophagic power of the whole blood of the patient as compared with that of the normal control, were all determined.

The blood of each of 3 cases of diphtheria was examined in this way at frequent intervals. In one, examined at the beginning of the attack, with many bacilli free in the secretions of the nose and throat and many inside leukocytes, the serum gave an opsonic index of 1.8, a cytophagic index of 4.2, and an opsono-cytophagic index of 4.3. For several weeks a few bacilli persisted in the nose and throat, while the indices fell, remaining at about 1.3, 1.8, and 1.8, respectively; then the indices went up to 3, 1.7, and 3.2 and the bacilli disappeared from the nose and throat. Two other acute cases with low indices showed many bacilli outside the leukocytes. In 2 days the bacilli disappeared at the same time as the indices went up, the latter remaining high until about the 10th day, when they returned to normal.

The blood of 10 diphtheria-carriers (Table 1) was found to have an increased phagocytic activity. In some in which the opsonic index was normal or slightly below, the activity of the leukocytes would be high, so that the total phagocytic power of the blood was well above normal.

It is evident from these results that in carriers the phagocytic power of the blood is increased, but that at the same time the nose and

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throat may contain large numbers of leukocytes not engaged in phagocytosis, diphtheria bacilli being found outside of the leukocytes. The question arose whether the virulence of the bacilli prevents phagocytosis. In these experiments the freshly isolated virulent diphtheria bacilli were readily phagocytable.

Various methods were then tried of increasing the activity of the leukocytes in the pharyngeal and nasal secretions. Fresh normal goat serum, which contains as much opsonin for diphtheria bacilli as is contained in normal human serum, was sprayed into the nose and throat every 2 hours for from 3 to 4 days, without, however, decreasing the number of diphtheria bacilli. This outcome suggested that possibly many of the leukocytes in the secretions were dead and incapable of taking part in phagocytosis. Next leukocytic suspensions were sprayed into the nose and throat every 2 hours, in the hope that fresh, actively phagocytic leukocytes might remove the bacilli, but seemingly this was also without effect.

TABLE 1

Phagocytosis in Diphtheria-Carriers

Carrier	Opsonic Index	Cytophagic Index	Opsono-cytophagic Index
1	6.4	8.0	6.0
	5.5	7.0	11.5
	1.7	2.2	2.1
2	2.0	1.5	1.5
	2.0	1.5	3.4
	1.5	1.9	3.0
3	1.2	2.1	6.7
4	3.9	0.8	4.3
	4.9	1.3	5.8
	5.0	3.3	5.8
5	2.0	3.5	6.5
6	0.5	1.7	1.7
	0.9	1.6	1.8
	1.1	3.7	2.6
7	2.0	3.5	6.5
8	1.0	1.9	1.7
9	2.0	2.3	2.8
10	3.8	6.5	7.8
	2.0	8.3	9.8
	2.8	2.3	4.7

Calcium chlorid, sodium salicylate, lactic acid, and magnesium chlorid, which have been found to increase phagocytosis, were tried also. The simplest method of testing the action of these chemicals on phagocytosis is to incubate for 15 minutes equal parts of the chemical to be tried, diluted with normal salt solution, serum, washed leukocytes, and bacteria—salt solution alone, being used in the controls. Experiments can be made also according to Hamburger's method, in which the mixtures of serum, leukocytes, and substances to be tested are kept at room temperature, then the bacteria or carbon particles or amylum, used by Hamburger, are added, and the whole incubated. My results were alike, strong solutions of the substances inhibiting, weak ones increasing, phagocytosis.

Experiments were also made to determine whether the action of the chemical substance was on the leukocytes themselves. Equal numbers of washed leukocytes were suspended in different dilutions of the chemical for from one-half to one hour, the leukocytes were then washed by centrifugation, serum and bacteria added, and the whole incubated for 15 minutes. The dilution at which chemicals affected phagocytosis varied in different experiments, as seen in Tables 2 and 3, the phagocytosis depending evidently upon the dilution of the leukocytes in the suspension. Human serum and leukocytes were generally employed in the experiments. Staphylococcus albus, Streptococcus pyogenes, diphtheria bacilli, and a spirillum were used in these tests, which were repeated many times. These tests were made in connection with a study on phagocytic activity, which is being continued.

Hamburger[1] showed that calcium chlorid in a 0.005% solution has a stimulating action on phagocytosis both in vitro and in vivo. He found that a certain mineral water and Ringer's solution, both containing calcium, also promoted phagocytosis. My experiments with crystallized calcium chlorid gave results which corresponded with Hamburger's.[2] Emmerich and Loew report the successful use of calcium chlorid in hay fever, basing its use on its physiologic action, including increased phagocytosis. They gave one teaspoonful at meals of a solution of 100 gm. of crystallized calcium chlorid in half a liter of distilled water. I used this method with diphtheria-carriers, and also sprayed into the nose and throat a 1:200 solution, without however, producing any effect on the number of bacilli.

Altho the salicylates are used so extensively in rheumatism and other acute infections, according to Hanzlik,[3] who reviews completely the literature on the subject, their therapeutic action is not understood. Miller[4] concluded that after absorption salicylic acid circulates and appears in the tissue as a salicylate; it cannot act as a germicide unless the increased carbonic acid tension in the joint, the result of inflammation, reconverts it into salicylic acid. According to Miller, statistics show that patients receiving salicylate are free from pain much earlier than those not so treated; that treated patients, however, relapse more frequently, so that the duration of pain and of the stay in the hospital of patients on salicylates is no shorter than in the case of patients receiving other forms of treatment, and that cardiac complications are not less frequent since the use of salicylates. He observed also that in rabbits the salicylate was of no value in preventing arthritis after intravenous injections of hemolytic streptococci. On the other hand, careful observers believe the salicylates to be of value for reasons other than their analgesic effect.

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[1] Untersuchungen über Phagocyten, 1912, p. 91.

[2] München. med. Wchnschr., 1915, 62, p. 43.

[3] Ann. Rep. Investigations of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the Am. Med. Assn., 1914, 3, p. 131.

[4] Jour. Am. Med. Assn., 1914, 63, p. 1107.

TABLE 2

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, HUMAN LEUKOCYTES, DIPHTHERIA BACILLI, AND SODIUM SALICYLATE

Dilution of Sodium Salicylate	Average Number of Bacilli in 50 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1: 100	0.48	24
1: 500	0.98	46
1: 1000	0.56	20
Salt solution	0.34	14

TABLE 3

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, HUMAN LEUKOCYTES, A SPIRILLUM, AND SODIUM SALICYLATE

Dilution of Sodium Salicylate	Average Number of Spirilla in 50 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1: 1000	0.72	28
1: 2000	0.86	36
1: 4000	0.96	46
1: 8000	0.66	34
Salt solution	0.38	24

Kentzler and v. Benczur,[5] in their work on the action of antipyretics on phagocytosis did not observe any stimulating effect of sodium salicylate on phagocytosis, but their dilutions of 1: 100 and 1: 1000 may have been too low for the number of leukocytes. Jacoby and Schütze,[6] experimenting with rabbits, found that sodium salicylate influenced the opsonic index, causing an increase in 9 of 12 rabbits examined. I found that sodium salicylate stimulated phagocytosis in dilutions of about 1: 1000 (Tables 2 and 3), and that the action was on the leukocytes themselves (Table 4). When strong solutions (1:5) were used, phagocytosis was inhibited. It is possible that the ability of sodium salicylate to promote phagocytosis may partly explain its beneficial action in rheumatism and other acute infections. To test these results 10-grain doses of sodium salicylate were given 3 times a day to diphtheria-carriers, without, however, any apparent effect on the number of bacilli in the nose and throat.

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[5] Ztschr. f. klin. Med., 1909, 67, p. 242.

[6] Biochem. Ztschr., 1908, 9, p. 527.

TABLE 4

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, SPIRILLA, AND LEUKOCYTES, WHICH HAD BEEN SUSPENDED FOR ONE-HALF HOUR IN SODIUM-SALICYLATE OR SALT SOLUTION AND THEN WASHED TWICE WITH SALT SOLUTION

Dilution of Sodium Salicylate	Average Number of Spirilla in 50 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1:1000	1.3	56
1:2000	1.2	52
1:4000	0.72	36
1:8000	0.64	36
Salt solution	0.64	38

TABLE 5

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, HUMAN LEUKOCYTES, STREPTOCOCCI, AND LACTIC ACID

Dilution of Lactic Acid	Average Number of Streptococci in 50 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1:20	0.04	2
1:50	2.9	42
1:100	4.1	82
1:200	3.9	68
1:400	2.4	62
1:800	2.6	56
Salt solution	2.3	60

Hektoen, quoted by Arkin,[7] found that lactic acid suspended phagocytosis in dilutions of 1:2000 and 1:4000. On the other hand, Bechhold[8] observed an increased phagocytosis with diluted lactic acid. The fact that their tests were made somewhat differently may account for the discrepancy between their results. I found that strong solutions of lactic acid suspended phagocytosis but that weak solutions (1:200) stimulated it (Table 5), and that the action was on the leukocytes themselves (Table 6). However, solutions of 1:200 applied locally in diphtheria-carriers, had no effect on the number of diphtheria bacilli. Local applications to the nose and throat are of course unsatisfactory because one cannot reach all parts. It is also possible that the dilutions used in the test-tube experiments are quite inadequate in vivo.

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[7] Jour. Infect. Dis., 1913, 13, p. 408.

[8] München. med. Wchnschr., 1908, 55, p. 1777.

Eggers[9] concluded that magnesium chlorid has some stimulating action on the leukocytes. Delbet's and Karajanopoulo's[10] experiments on dogs and in vitro appear to point out what they call the "cytophylactic power" of a 12.1:1000 solution of anhydrous magnesium chlorid. They found it useful in dressing wounds and in subcutaneous injections. Pinard[11] used 18 gm. of crystallized magnesium chlorid to 1000 c.c. of sterilized water for irrigating wounds and found that healing occurred far more rapidly than with any other treatment. The results of my experiments with magnesium chlorid show that it stimulates phagocytosis considerably (Table 7). Used as a spray in a dilution of 18 gm. of the crystallized salt to 1000 c.c. of normal salt solution, in a diphtheria-carrier, it was without effect in reducing the number of bacilli, altho smears showed more phagocytosis after the spraying than before.

TABLE 6

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, STREPTOCOCCI, AND LEUKOCYTES, WHICH HAD BEEN SUSPENDED IN LACTIC ACID (1: 200) OR SALT SOLUTION FOR ONE HOUR AND THEN WASHED TWICE WITH SALT SOLUTION

Dilution of Lactic Acid	Average Number of Streptococci in 50 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1: 200	11.3	64
Salt solution	5.6	40

TABLE 7

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, HUMAN LEUKOCYTES, STREPTOCOCCI, AND MAGNESIUM CHLORID

Dilution of Magnesium Chlorid	Average Number of Streptococci in 50 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1: 41	2.4	26
1: 82	2.7	26
1: 164	3.5	42
1: 328	3.8	40
1: 656	2.5	30
1: 1312	1.3	20
Salt solution	0.88	18

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[9] Jour. Infect. Dis., 1909, 6, p. 662.

[10] Bull. de l'Acad. de méd., 1915, 74, p. 266.

[11] Ibid., p. 577.

SUMMARY

The phagocytic power of the blood of diphtheria-carriers is increased, but at the same time the nose and throat may contain large numbers of leukocytes not engaged in phagocytosis. Efforts to increase the activity of these leukocytes by the use of normal serum, leukocytic suspensions, calcium chlorid, sodium salicylate, lactic acid, and magnesium chlorid were unsuccessful, altho dilute solutions of these substances are found to promote phagocytosis in the test tube.